

Phytopathol. Mediterr. (2003) 42, 9–16

Effect of salicylic acid on phenolic compounds related to date palm resistance to *Fusarium oxysporum* f. sp. *albedinis*

ABDELHI DIHAZI, FATIMA JAITI, JAMILA ZOUINE, MAJIDA EL HASSNI and ISMAIL EL HADRAMI

Laboratoire de Physiologie Végétale, Equipe Biotechnologie et Physiologie Végétales, Département de Biologie, Faculté des Sciences Semlalia, B.P 2390, 40 000 Marrakesh, Morocco

Summary. Salicylic acid (SA) plays a key role in establishing resistance to pathogens in many plants. To study the possible involvement of SA in the resistance of date palm (*Phoenix dactylifera* L.) to *Fusarium oxysporum* f. sp. *albedinis* (FOA), we investigated levels of phenolic compounds, known as indicators of resistance in the date palm/*Fusarium* pathosystem. After treatment with SA the content of root soluble phenolics in *F. oxysporum* inoculated date palm seedlings was about 4 times higher in cv. Bousthami noir and 6 times higher in cv. Jihel than that in untreated plants showing disease symptoms. The largest increase was at a SA concentration of 50 μ M. SA treatment also enhanced the content of cell wall phenolics. In addition, inoculation of SA-treated roots of date palm with FOA (strain ZAG) resulted in a greater number of plants showing only limited hypersensitive reaction-like necrotic lesions. In contrast, SA-untreated plants normally showed spreading necrosis in response to fungus inoculation.

Key words: bayoud, defence mechanisms, *Phoenix dactylifera*, Fusarium wilt, phytoalexins.

Introduction

Plants have evolved numerous mechanisms to defend themselves against pathogens. Some of these mechanisms are constitutive, while others are induced in response to pathogen attack. These induced defence mechanisms are activated at infection site, and manifest themselves in part as necrotic lesions (hypersensitive response, HR). Induced resistance also occurs in the distal uninfected parts of infected plants, leading to systemic acquired resistance (SAR) (Goodman and Novacky, 1994). Salicylic acid (SA) has been recognised as an important signal molecule indicating that plant

defence has been activated against pathogens (Enyedi *et al.*, 1992; Gaffney *et al.*, 1993). SAR and local resistance are generally accompanied by higher levels of endogenous SA (Malamy and Klessig, 1992; Dorey *et al.*, 1997). SAR can also be induced by exogenous application of SA (Kessmann *et al.*, 1994; Lawton *et al.*, 1996). In tobacco, the same defence genes that were expressed upon infection with tobacco mosaic virus were also activated by treatment with SA (Ward *et al.*, 1991). More recent results have shown that transgenic tobacco plants expressing bacterial salicylate hydroxylase, which metabolises SA, are unable to induce SAR, and even show enhanced susceptibility to pathogens (Gaffney *et al.*, 1993). At the same time phenol metabolism is activated in plants reacting to pathogens (Ellard-Ivery and Douglas, 1996). This is due not only to the mechanical role that phenolics play in cell walls, but also to their anti-

Corresponding author: I. El Hadrami
Fax: +212 44 439997
E-mail: hadrami@ucam.ac.ma

fungal properties (Harborne, 1991). New phenolics are synthesised in date palms (*Phoenix dactylifera* L.) following infection with *Fusarium oxysporum* f. sp. *albedinis* (FOA), the agent causing bayoud disease (El Hadrami *et al.*, 1997). However, little is known about the effect of SA on phenol metabolism in relation to the resistance of date palm to FOA.

The aim of this work was to determine the effect of SA treatment on phenol metabolism in relation to date palm resistance to FOA.

Materials and methods

Fungal and plant material

Fusarium oxysporum f. sp. *albedinis* conidia were harvested from infected date palm leaves brought from Zagora in the south of Morocco and germinated on malt-agar at 25°C in darkness. The inoculum was a conidial suspension of the ZAG strain known for its aggressiveness (El Idrissi Tourane *et al.*, 1995). The conidial suspension was adjusted to 6×10^6 spores ml⁻¹. Three-month-old date palm seedlings were obtained from seeds produced by mother cultivars highly susceptible to FOA wilt (bayoud). They were grown in pots containing sand (75%) and peat (25%) in a greenhouse at 30°C, under a 16 h light/8 h darkness regime. The seedling roots of two date palm cultivars, susceptible Jihel (JHL) and resistant Bousthami noir (BSTN), were infected by injecting 10 µl of conidial suspension into the roots.

Effect of SA treatment on the content of soluble phenolics

In two separate experiments, the roots of three-month-old date palm seedlings of both cultivars were soaked in SA at various concentrations (1 mM, 0.2 mM, 0.05 mM). Control plants were treated with sterile distilled water. For the soluble phenolic contents, 30 date palm seedlings per cultivar were used at each SA concentration; an equal number of plants was used for the control. Each value is the mean of two replicates (400 mg f wt of roots from 5 seedlings per replicate).

Effect of SA treatment on the survival of date palm seedlings

One hundred seedlings of each cultivar were treated with SA (50 µM) by soaking their roots in

SA solution for 10 days before inoculation with one of the conidial suspensions. The control plants were inoculated but not treated with SA. The survival of seedlings was recorded one month after inoculation. The results were the mean of two repetitions with 50 plants for each repetition. The phenolic content of the seedling roots was also determined.

Extraction and quantification of soluble phenolic compounds

Levels of soluble phenolic compounds were determined according to Ziouti *et al.* (1992). Root tissues (400 mg from 5 plants), taken from around the infection sites, were homogenised in an ice bath with 2 ml (80%) methanol. The homogenate was centrifuged three times at 7000 g for 3 min; supernatants were recuperated each time. One hundred microlitres of the supernatant was added to Folin-Ciocalteu reagent and sodium carbonate. The mixture was incubated at 40°C for 30 min and the blue colour was read at 760 nm. The content of soluble phenolic compounds was determined for the experiments described above; it was expressed in mg-equivalents of catechin per g of f wt.

Extraction and quantification of cell wall phenolic compounds

The residue of tissue after soluble phenol extraction was washed three times with methanol and then incubated in the presence of 6N HCl at 100°C for 2 h. The cell wall phenolic compounds were extracted three times with diethyl ether. After evaporation of the diethyl ether, the phenolics were resuspended in 200 µl methanol. The cell wall phenolic content was determined as described for soluble phenolics.

Chromatographic analysis by TLC and HPLC

TLC was conducted on microcrystalline cellulose plates in the upper phase of BAW (butanol-acetic acid-water, 4:1:5 [v:v:v]). UV was used for phenolic detection with and without NH₃ vapour.

Phenolic compounds were separated and identified by HPLC (El Hadrami *et al.*, 1997) with a Waters 600 E liquid chromatograph (Waters Millennium, En Yvelines Cedex, France) equipped with a Waters 990 photodiode array detector.

Results

Effect of SA treatment on the survival of date palm seedlings

SA treatment enhanced the resistance of date palm to *FOA*. Without SA treatments, diseased control plants showed progressing wet necrosis of the roots earlier in the susceptible JHL cv. (4 days) than in the resistant BSTN cv. (8 days). One month after inoculation, 51% of the plants of the susceptible cultivar and 21% of the resistant cultivar had completely collapsed. In contrast, one month after SA treatment 24% of plants of cv. JHL and 9% of plants of cv. BSTN had died from *Fusarium wilt* (Table 1). SA-treated plants without disease symptoms generally developed limited necrotic lesions, which reflected a delay in the colonisation of the host plant by the pathogen.

Effect of SA on soluble phenolic compounds

The relation between SA and soluble phenolic compounds following SA treatments at 50 μ M, 200 μ M and 1 mM was studied in the roots of the date palm cv. JHL and BSTN. For cv. JHL (Fig. 1), treatment with a low SA concentration (50 μ M) en-

Table 1. Effect of salicylic acid (50 μ M) treatment on the survival of seedlings of two cultivars of date palm, one month after inoculation with *Fusarium oxysporum* f. sp. *albedinis*. Values are the mean of two repetitions, 50 plants per repetition. The results obtained were significantly different. $SE \leq 5\%$.

Cultivar	Survival (%)	
	Control	SA-treated
Jihel	49	76
Bousthami noir	80	91

hanced the content of soluble phenols with two peaks, 0.62 and 0.65 mg per g of f wt, respectively 3 and 12 days after treatment. A higher SA concentration (1 mM) decreased phenol content, to 0.24 and 0.2 mg per g of f wt at 3 and 12 days after treatment respectively. With cv. BSTN (Fig. 2), no significant differences were detected between SA-treated plants and the controls. However, higher SA concentrations (200 μ M and 1 mM) slightly inhibited the levels of soluble phenolics.

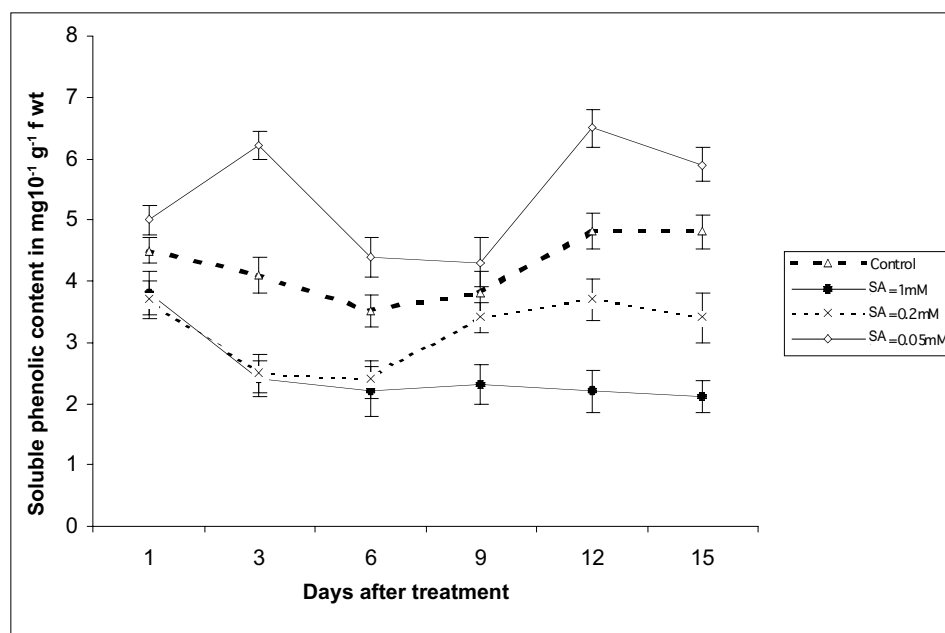


Fig. 1. Time-course of changes in the content of free phenolics in roots of date palm cv. Jihel in response to different SA concentrations. Each bar is the mean of two replicates root extracts.

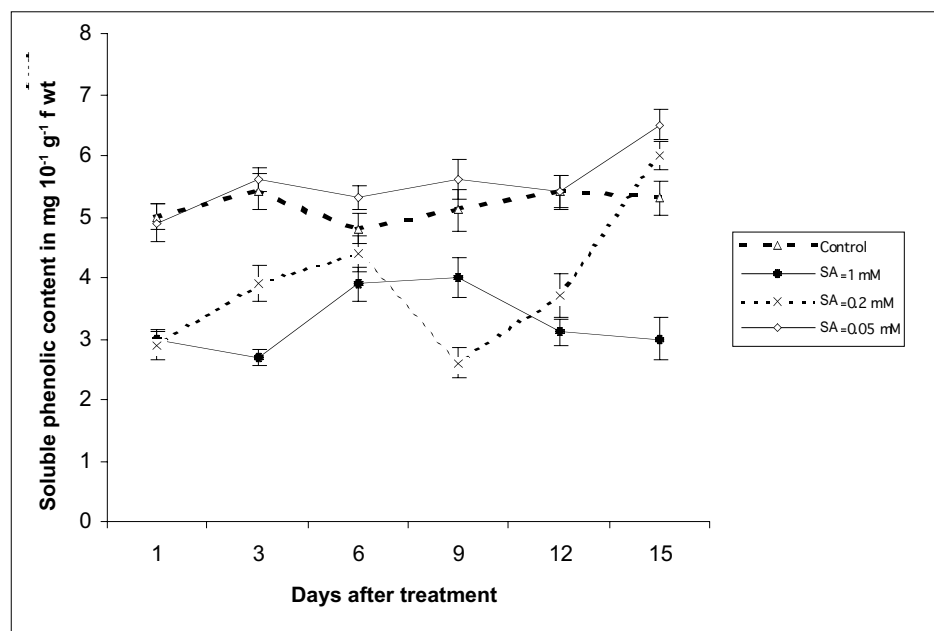


Fig. 2. Time-course of changes in the content of free phenolics in roots of date palm cv. Bousthami noir in response to different SA concentrations. Each bar is the mean of two replicates root extracts.

Effect of SA treatment on the soluble phenols content in date palm roots inoculated with *F. oxysporum* f. sp. *albedinis*

There was a relationship between the level of soluble phenolic compounds in the roots of both cultivars and the survival rate of SA-treated and -untreated plants. The level of soluble phenolics in roots was much higher in SA-treated plants than in symptomless plants infected with *FOA* (Fig. 3). Indeed, the content of soluble phenolics in infected roots of SA-treated cultivar JHL showing no symptoms was $3.5 \text{ mg g}^{-1} \text{ f wt}$. This was about 2 times higher than that found in untreated infected plants without disease symptoms and 6 times higher than that in untreated and inoculated plants with disease symptoms.

For date palm cv. BSTN, the content of root soluble phenolics in infected SA-treated plants was $2.5 \text{ mg g}^{-1} \text{ f wt}$. For infected plants showing no symptoms (Fig. 3), this was about 2 times higher than that found in untreated infected plants without symptoms and 4 times higher than that found in untreated inoculated plants with symptoms.

Establishment of resistance was associated with the formation of HR-like lesions around the point

of inoculation of *FOA*. In contrast, inoculated untreated plants showed spreading necrosis.

Inoculation of date palm roots stimulated the phenol metabolism of symptomless infected plants of both cultivars; this metabolism was greater when SA treatment was given, and was more evident for susceptible JHL than for resistant BSTN cultivar.

SA treatment (0.05 mM) and cell wall phenolics in date palm roots inoculated with *F. oxysporum* f. sp. *albedinis*

The content of cell wall phenolic compounds was greater in the resistant than in the susceptible cultivar. This content showed little increase in either cultivar after inoculation with *FOA*. Furthermore, the level of cell wall phenolics induced by SA did not significantly differ from that in the untreated controls (Fig. 4).

Characterisation of soluble phenolic compounds in SA-treated date palm roots after inoculation with *F. oxysporum* f. sp. *albedinis*

Analysis by TLC and HPLC showed a relationship between resistance to *FOA* and the level of new soluble phenolic compounds. The control roots

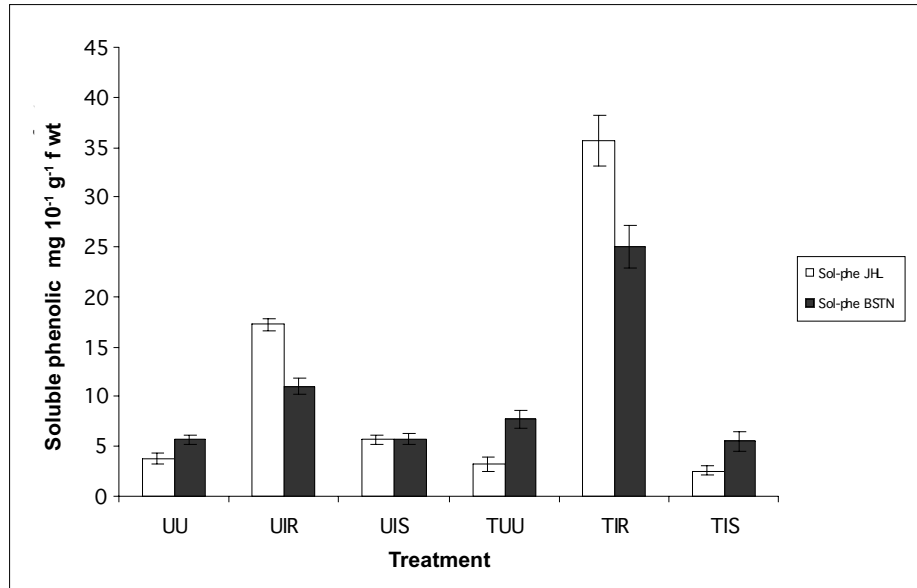


Fig. 3. Effect of SA treatment (50 μ M) on soluble phenolic contents in roots of date palm cv. Jihel (JHL) and cv. Bousthami noir (BSTN) one month after inoculation with *Fusarium oxysporum* f. sp. *albedinis*. UU (uninoculated plants); UIR (untreated and inoculated plants without disease symptoms); UIS (untreated and inoculated plants with disease symptoms); TUU (treated and uninoculated plants); TIR (treated and inoculated plants without disease symptoms); TIS (treated and inoculated plants with symptoms). Each bar is the mean of two replicate root extracts. Sol-phe, soluble phenolics

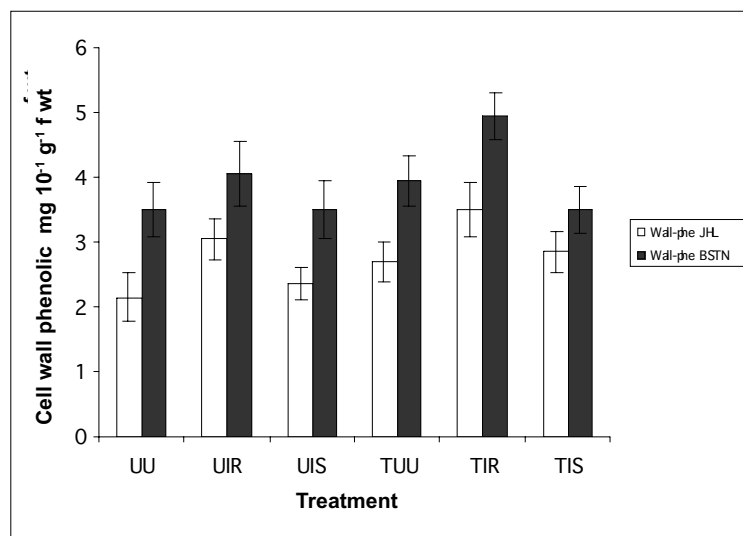


Fig. 4. Effect of SA treatment (50 μ M) on cell wall phenolics in roots of date palm cv. Bousthami noir (BSTN) and cv. Jihel (JHL) one month after inoculation with *Fusarium oxysporum* f. sp. *albedinis*. UU (untreated and uninoculated plants); UIR (untreated and inoculated plants without disease symptoms); UIS (untreated and inoculated plants with disease symptoms); TU (treated and uninoculated plants); TIS (treated and inoculated plants with symptom disease); TIR (treated and inoculated plants without disease symptoms). Each bar is the mean of two replicate root extracts. Wall-phe, cell wall phenolics.

had higher levels of caffeoylshikimic acids (Fig. 5, left). When infected, the phenolic profiles of the roots changed completely. Several new non-constitutive compounds now accumulated. They were hydroxycinnamic derivatives, revealed at 320 nm, and mainly represented in this experi-

ment by a *p*-coumaric derivative (pic 1) and a sinapic derivative (pic I₂) (Fig. 5, right). The phenolics that accumulated in infected plants and those in infected SA-treated plants were the same, and they were associated with the establishment of resistance.

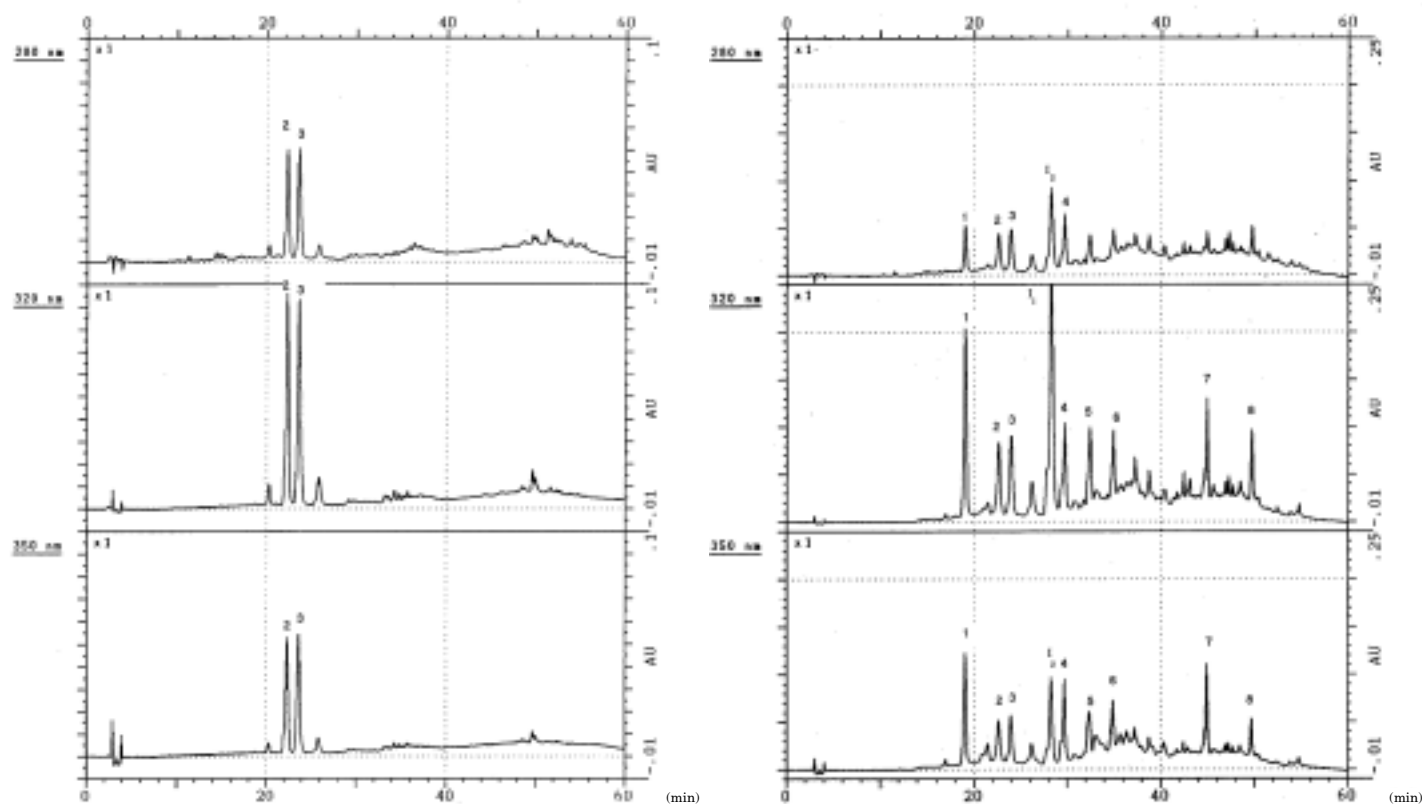


Fig. 5. Typical HPLC profiles obtained with three wave length (280 nm, 320 nm and 350 nm) of phenolics in root extracts of date palm before (left) and after (right) inoculation with *Fusarium oxysporum* f. sp. *albedinis*. 1: *p*-coumaric derivative; 2, 3: caffeoylshikimic acids, I₂: sinapoyl-amide; 4, 5, 6, 7 and 8: other hydroxycinnamic derivatives.

Discussion

SA treatment increased the resistance of seedlings to *Fusarium* wilt. This is in agreement with other reports showing that the treatment of plants with SA can provide a significant degree of protection against pathogens (Métraux *et al.*, 1990; Kauss *et al.*, 1992; Raskin, 1992; Gaffney *et al.*, 1993; Van Kan *et al.*, 1995). This study showed that the lower susceptibility of date palm to *FOA* that was in-

duced by SA was associated with the formation of HR-like lesions around the point of infection. Normally, hypersensitive lesions occur during an incompatible interaction. However, it has been reported that plants in a state of SAR resist to virulent fungal pathogens with single-cell HR (Kogel *et al.*, 1994). Inoculation of SA-treated date palm roots with aggressive *FOA* strain ZAG caused a higher number of plants with such HR-like necrotic lesions. The HR is associated with cell death and

restricts fungal colonisation by synthesis of chemical barriers against pathogen spread (Hammond-Kosack *et al.*, 1996; Hammerschmidt, 1999). As well as delaying the development of disease, SA also affected the accumulation of phenolic compounds which are themselves likewise associated with plant resistance (Nicholson and Hammerschmidt, 1992; Métraux and Raskin, 1993; El Hadrami *et al.*, 1997; Mansfield, 1999). At higher SA concentration (1 mM), the level of soluble phenolics in date palm roots decreased; this reduction was already reported by Dixon and Paiva (1995). SA may inhibit the activity of PAL, a key enzyme in the synthesis of phenolic compounds (Nicholson and Hammerschmidt, 1992). At low concentrations, SA stimulated the accumulation of soluble phenolic compounds in date palm roots. It has been suggested that SA inhibits catalase activity, leading to increased levels of H_2O_2 (Chen *et al.*, 1993), which in turn induces PAL gene expression (Desikan *et al.*, 1998) and synthesis of phenolic compounds (Dorey *et al.*, 1997). The study also showed that SA treatment greatly increased the content of soluble phenolics in inoculated date palm root compared with uninoculated plants. This is in agreement with Kauss *et al.* (1992), who reported that SA did not directly induce the expression of resistance genes, but rather increased the sensitivity of cells to subsequent elicitor treatment. In cucumber cotyledons, it was also found that SA increased the ability of a tissue to trigger a burst of H_2O_2 in response to subsequent elicitor treatment (Kauss and Jeblick, 1995; Fauth *et al.*, 1996). In addition, it was shown that levels of soluble phenolics were higher in the symptomless parts of the susceptible SA-treated cv. JHL than in the resistant BSTN. These higher levels are likely to be associated with the establishment of resistance since phenolic compounds have been found to have anti-fungal properties in several host-parasite interactions (Harborne, 1991). Moreover, treatment with SA slightly increased the content of cell wall phenolics. The accumulation of cell wall phenolics clearly distinguished the cultivars tested being faster and more intense in the resistant cultivar than in the susceptible one. Such an accumulation is often associated with plant resistance (Niemann *et al.*, 1991; Ikegawa *et al.*, 1996). The insolubilisation of phenolic compounds in the cell walls constitutes a mechanical defence barrier protecting the wall

against cell wall-degrading enzymes (Matern and Grimmig, 1993). In addition, we found that in both the susceptible and the resistant cultivar tested, infected SA-treated roots showed the same soluble phenolics as those that occur in untreated symptomless plants. These phenolics were different from those expressed by uninoculated plants. In summary, the results indicate that SA protects date palm from *Fusarium* wilt and suggest that SA induces date palm resistance by enhancing induced chemical defences.

Acknowledgements

Financial support for this work by the French-Moroccan cooperation (PRAD 00-05; 02-02 / AI: MA/01/23) and CNRST- Morocco: PROTARS II/ P51-14 is gratefully acknowledged.

Literature cited

- Chen Z., S. Silva and D. Klessig, 1993. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science* 262, 1883–1886.
- Desikan R., A. Reynolds, J.T. Hancock and S.J. Neill, 1998. Harpin and hydrogen peroxide both initiate programmed cell death but have differential effects on defence gene expression in Arabidopsis suspension cultures. *Biochemistry Journal* 330, 115–120.
- Dixon R. and N. Paiva, 1995. Stress induced phenyl-propanoid metabolism. *The Plant Cell* 7, 1085–1097.
- Dorey S., F. Baillieul, M.A. Pierrel, P. Saindrenan, B. Fritig, and S. Kauffmann, 1997. Spatial and temporal induction of cell death, defence genes, and accumulation of salicylic acid in tobacco leaves reacting hypersensitively to a fungal glycoprotein elicitor. *Molecular Plant-Microbe Interactions* 11, 1102–1109.
- El Hadrami I., T. Ramos, M. El Bellaj, A. El Idrissi-Tourane and J.J. Macheix, 1997. A sinapic derivative as induced defence compound of date palm against *Fusarium oxysporum* f. sp. *albedinis*, the agent causing bayoud disease. *Journal of Phytopathology* 145, 329–333.
- El Idrissi-Tourane A., A. Oihabi and I. El Hadrami, 1995. Effet des polyamines et des inhibiteurs de leur biosynthèse sur la croissance mycélienne de *Fusarium oxysporum* f. sp. *albedinis* responsable du bayoud chez le palmier dattier. *Revue du Réseau d'Amélioration de la Production Agricole en Milieu Aride* 7, 241–247.
- Ellard-Ivery M. and C.J. Douglas, 1996. Role of jasmonates in the elicitor and wound-inducible expression of defence genes in parsley and transgenic tobacco. *Plant Physiology* 112, 183–192.
- Enyedí A.J., N. Yalpani, P. Silverman and I. Raskin, 1992. Localisation, conjugation and function of salicylic acid in tobacco during the hypersensitive reaction to tobacco

- co mosaic virus. *Proceedings of the National Academy of Sciences USA* 89, 2480–2484.
- Fauth M., A. Merten, M.G. Hahn, W. Jeblick and H. Kauss, 1996. Competence for elicitation of H₂O₂ in hypocotyls of cucumber is induced by breaching the cuticle and is enhanced by salicylic acid. *Plant Physiology* 110, 347–354.
- Gaffney T., L. Friedrich, B. Vernooij, D. Nergotto, G. Nye, S. Uknes, E. Ward, H. Kessmann, and J. Ryals, 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* 261, 754–756.
- Goodman R. and A. Novacky, 1994. *The Hypersensitive Reaction in Plants to Pathogens: A Resistance Phenomenon*. APS Press, St Paul, MN, USA, 244 pp.
- Hammerschmidt R., 1999. Induced resistance: how do induced plants stop pathogens? *Physiological and Molecular Plant Pathology* 55, 77–84.
- Hammond-Kosack K.E. and J.D.G. Jones, 1996. Resistance gene-dependent plant defense responses. *Plant Cell* 8, 1773–1791.
- Harborne J.B., 1991. Role of secondary metabolites in chemical defence mechanisms in plants. In: *Bioactive Compounds from Plants*. (D.J. Chadwick, J. Marsh, ed.), John Wiley and Sons, Chichester, UK, 126–139.
- Ikegawa T., S. Mayama, H. Nakayashiki and H. Kato, 1996. Accumulation of diferulic acid during the hypersensitive response of oat leaves to *Puccinia coronata* f. sp. *avenae* and its role in the resistance of oat tissues to cell wall degrading enzymes. *Physiology and Molecular Plant Pathology* 48, 245–256.
- Kauss H., E. Theisinger-Hinkel, R. Mindermann and U. Conrath, 1992. Dichloroisonicotinic and salicylic acid, inducers of systemic acquired resistance, enhance fungal elicitor responses in parsley cells. *Plant Journal* 2, 655–660.
- Kauss H. and W. Jeblick, 1995. Pretreatment of parsley suspension cultures with salicylic acid enhances spontaneous and elicited production of H₂O₂. *Plant Physiology* 108, 1171–1178.
- Kessmann H., T. Staub, C. Hofmann, T. Maetzke and J. Herzog, 1994. Induction of systemic acquired disease resistance in plants by chemicals. *Annual Review of Phytopathology* 32, 439–459.
- Kogel K.H., U. Beckhove, J. Dreschers, S. Munch and Y. Romme, 1994. Acquired resistance in barley. *Plant Physiology* 106, 1269–1277.
- Lawton K., L. Friedrich, M. Hunt, K. Weymann, T. Staub, H. Kessmann and J. Ryals, 1996. Benzothiadiazole induces disease resistance in Arabidopsis by activation of the systemic acquired resistance signal transduction pathway. *Plant Journal* 10, 71–82.
- Malamy J. and D. Klessig, 1992. Salicylic acid and plant defense resistance. *Plant Journal* 2, 643–654.
- Mansfield J.W., 1999. Antimicrobial compounds and resistance: the role of phytoalexins and phytoanticipins. In: *Mechanisms of Resistance to Plant Diseases*. (A.J. Slusarenko, R.S.S. Fraser, L.C. Van Loon ed.), Kluwer, Amsterdam, Netherlands.
- Matern U. and B. Grimmig, 1993. Polyphenols in plant pathology. In: *Polyphenolic Phenomena* (A. Scalbert, ed.), INRA Editions, Paris, France, 143–147.
- Métraux J.P. and I. Raskin, 1993. Role of phenolics in plant disease resistance. In: *Biotechnology in Plant Disease Control*. Wiley-Liss Inc., New York, NY, USA 191–209.
- Métraux J.P., H. Signer, J. Ryals, E. Ward, M. Wyss-Benz, J. Gaudin, K. Raschdorf, E. Schmid, W. Blum and B. Inverardi, 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250, 1004–1006.
- Nicholson R.L. and R. Hammerschmidt, 1992. Phenolic compounds and their role in disease resistance. *Annual Review of Phytopathology* 30, 369–371.
- Niemann G.J., A. Van Der Kerv, W.M.A. Niessen and K. Versluis, 1991. Free and cell wall-bound phenolics and other constituents from healthy and fungus-infected carnation (*Dianthus caryophyllus* L.) stems. *Physiology and Molecular Plant Pathology* 38, 417–432.
- Raskin I., 1992. Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 43, 439–463.
- Van Kan J.A.L., T. Cozijnsen, N. Danhash and P.J.G.M. De Wit, 1995. Induction of tomato stress protein mRNAs by ethephon, 2,6-dichloroisonicotinic acid and salicylate. *Plant Molecular Biology* 27, 1205–1213.
- Vernooij B., L. Friedrich, A. Morse, R. Reist, R. Kolditz-Jahwar, E. Ward, S. Uknes, H. Kessmann and J. Ryals, 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *Plant Cell* 6, 959–965.
- Ward E.R., S. Uknes, S.C. Williams, S.S. Dincher, D.L. Wiederhold, D.C. Alexander, P. Ahl-Goy, J.P. Métraux and J.A. Ryals, 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3, 1085–1094.
- Ziouti A., C. El Modafar, A. Fleuriet, S. El Boustani and J.J. Macheix, 1992. Les polyphénols, marqueurs potentiels de la résistance du palmier dattier (*Phoenix dactylifera* L.) au *Fusarium oxysporum* f. sp. *albedinis*. *Compte rendu du Groupe Polyphénols* 16, 346–349.

Accepted for publication: November 11, 2002